PATENT ABSTRACTS OF JAPAN

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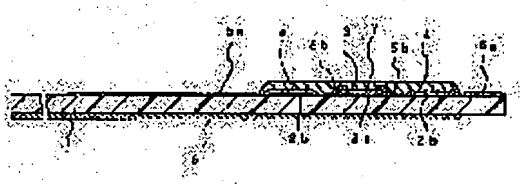
(54) SUBSTRATE QUANTITATIVE DETERMINING METHOD, AND BIOSENSOR AND MEASURING DEVICE USED THEREFOR

(57)Abstract:

PROBLEM TO BE SOLVED: To make the substrate concentration of a liquid sample stably quantitatively determinable without being influenced by temperature by reacting an enzyme with a substrate in a sample to be tested while heating and holding a reaction reagent layer to a specified temperature.

SOLUTION: When a glucose sensor containing glucose oxidase and potassium ferricyanide respectively as enzyme and an electron acceptor in a reaction reagent layer 7 is mounted on a measuring device, a heater is actuated, and its heat raises the temperature of the reaction reagent layer 7 through a heat transfer body 6. The measuring device is controlled to heat and hold the sensor to a specified temperature, and when the sensor reaches the specified temperature, a sample to be tested is fed to the reaction reagent layer 7. The reaction reagent layer 7 dissolves, and glucose contained in the sample is oxidized by glucose oxidase to become gluconic acid, while potassium ferricyanide is reduced to become potassium ferrocyanide. When a voltage is applied between electrodes, potassium ferrocyanide is electrolytically oxidized, and the value obtained by measuring an oxidation



current flowing to a measuring electrode 3 is compared with an analytical curve to judge glucose concentration of the sample.

LEGAL STATUS

[Date of request for examination]

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CLAIMS

[Claim(s)]
[Claim 1] An insulating substrate and the electrode system which was formed on said insulating substrate and which contains the electrode of a pair at least, the biosensor possessing the reaction reagent layer containing the enzyme formed in contact with said electrode system -- using -- said reaction reagent layer -- warming -- with the process to which supply the specimen which contains a substrate in said reaction reagent layer, and said substrate and said enzyme are made to react, holding Assay of the substrate possessing the process which carries out the quantum of the substrate in said specimen based on the amount of currents which impresses an electrical potential difference to inter-electrode [said], and flows to either of said electrodes.
[Claim 2] Assay of a substrate according to claim 1 with which said enzymes are said substrate and an oxidoreductase to which it reacts specifically, and said reaction reagent layer includes an electron acceptor further.
[Claim 3] a reaction reagent layer including an enzyme and an electron acceptor -- warming -- the assay of the substrate possessing the process which supplies the specimen which contains a substrate in said reaction reagent layer while holding, and the process which detects change of the absorbance of said reaction reagent layer.
[Claim 4] said enzyme -- glucose oxidase -- it is -- said reaction reagent layer -- 30-50 degrees C -- warming -- the assay of the substrate according to claim 1 or 3 to hold.
[Claim 5] warming for warming an insulating substrate, the electrode system which was formed on said insulating substrate and which contains the electrode of a pair at least, the reaction reagent layer containing the enzyme formed in contact with said electrode system, and said reaction reagent layer -- the biosensor possessing the section.
[Claim 6] said warming -- the biosensor according to claim 5 which the section is a heat transfer object for telling the heat from an external heat source to said reaction reagent layer, and was formed in the field opposite to the field equipped with the periphery of said reaction reagent layer, or the reaction reagent layer of said substrate.
[Claim 7] said warming -- the biosensor according to claim 5 with which the section contains a metal.
[Claim 8] The biosensor according to claim 5 with which said enzymes are said substrate and an oxidoreductase to which it reacts specifically, and said reaction reagent layer includes an electron acceptor further.
[Claim 9] The measuring device which is the measuring device which carries out the quantum of the substrate in a specimen using the biosensor possessing an insulating substrate, the electrode system which was formed on said insulating substrate, and which contains the electrode of a pair at least, and the reaction reagent layer containing the enzyme formed in contact with said electrode system, and possesses a means impress an electrical potential difference to inter-electrode [said], a means detect the amount of currents which flows to said electrode, and a means warm said reaction reagent layer.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the assay of the substrate in a sample.
[0002]
[Description of the Prior Art] As a means which carries out the quantum of the substrate in a sample quickly and with high precision, there is a biosensor indicated by JP,7-114705,B, for example. The biosensor indicated by this official report is shown in drawing 5 . The electrode system which consists of the measurement pole 12, a counter electrode 13, and a reference pole 10 is formed in the front face of the insulating substrate 11. The reaction reagent layer 14 is formed so that these electrode system may be touched. The reaction reagent layer 14 includes a hydrophilic macromolecule, an oxidoreductase, and an electron acceptor. After this biosensor forms the measurement pole 12, a counter electrode 13, and the reference pole 10 on a substrate 11 by approaches, such as screen-stencil, it forms the insulating layer 15 which has opening of predetermined area, and forms the reaction reagent layer 14 in this opening.
[0003] This biosensor is the following, and is made and used. The sample solution containing the substrate which it is going to measure is supplied to the reaction reagent layer 14. The reaction reagent layer 14 dissolves by this, and a substrate oxidizes with an oxidoreductase further. At this time, the electron acceptor included in the reaction reagent layer 14 is returned. From supply of a sample solution, an electrical potential difference is impressed between the measurement pole 12 and a counter electrode 13 after predetermined time progress, and this returned electron acceptor is oxidized electrochemically. It measures at this time, the amount of currents of oxidation current, i.e., amount, which flows to the measurement pole 12. The relational expression of substrate concentration and the amount of oxidation current is beforehand memorized as a calibration curve by the measuring device, and a measuring device computes the substrate concentration in a sample solution for the obtained amount of oxidation current to it as compared with this calibration curve. The measurement to various matter is possible for such a biosensor by choosing as arbitration the enzyme which makes the matter used as the measuring object a substrate. However, the amount of oxidation current measured as mentioned above is influenced by the degree of advance of the enzyme reaction of the sample in the time. Since advance of an enzyme reaction is greatly dependent on the temperature of a reaction place, the value of the substrate concentration obtained by the above approaches is changed with the room temperature at the time of measuring, or the temperature of a sample solution.
[0004]
[Problem(s) to be Solved by the Invention] This invention aims at offering the quantum approach of the substrate which is stabilized and can carry out the quantum of the substrate concentration of a sample solution, without solving the above trouble and being influenced of environmental temperature or the temperature of a sample solution.
[0005]
[Means for Solving the Problem] the place, i.e., the reaction reagent layer, which performs an enzyme

reaction according to this invention -- fixed temperature -- warming -- the substrate and enzyme in a specimen are made to react, holding

[0006]

[Embodiment of the Invention] The electrode system by which the assay of the substrate of this invention was formed on the insulating substrate and the insulating substrate and which contains the electrode of a pair at least, the biosensor possessing the reaction reagent layer containing the enzyme formed in contact with the electrode system -- using -- a reaction reagent layer -- warming -- with the process to which supply the specimen which contains a substrate in a reaction reagent layer, and a substrate and an enzyme are made to react, holding inter-electrode -- an electrical potential difference -- impressing -- the electrode of a pair -- the process which carries out the quantum of the substrate in a specimen based on the amount of currents which flows to inner either is provided. In the assay of the substrate using an enzyme reaction, the specific activity of an enzyme is changed with the temperature of a reaction place. Therefore, the temperature of a reaction place has big effect on the precision of a quantum. If the temperature of a reaction place is low, an enzyme reaction rate will become slow, and if temperature is high, an enzyme reaction rate will tend to become quick. Then, in the case of measurement, an enzyme reaction place, i.e., a reaction reagent layer, is warmed, and specific activity of an enzyme is made high. If the specific activity of an enzyme becomes high, a reaction rate will become large and the accuracy of measurement will improve remarkably. Moreover, it also becomes possible to shorten the detection time of a sensor.

[0007] especially -- a reaction reagent layer -- specific temperature -- warming -- an enzyme reaction can always be advanced at a fixed rate, without being influenced of the environment measured by holding, or the temperature of a specimen. Thereby, the variation in the measured value resulting from a temperature gap can be controlled. Preferably, it is the temperature to which the specific activity of an enzyme becomes high about the temperature of a reaction reagent layer, for example, a glucose sensor, and when using glucose oxidase for an enzyme, it is desirable to warm at 30-50 degrees C. After the above-mentioned assay makes a substrate react with an enzyme in a reaction reagent layer, it can be used for the approach of detecting the amount of the oxidation current which impresses the suitable electrical potential difference for the matter generated by the enzyme reaction, and is then acquired, or reduction current, or its quantity of electricity. Especially, enzymes are a substrate and an oxidoreductase to which it reacts specifically, and if it uses for the assay using the biosensor of the type to which an electron acceptor is made to return while oxidizing the biosensor with which a reaction reagent layer includes an electron acceptor further, i.e., the substrate which it is going to measure by the enzyme reaction, high measurement of precision will be attained more. In addition, it is also possible to use for the approach of detecting change of the absorbance accompanying the oxidation reduction reaction of the electron acceptor which the reaction reagent layer was made containing. In this case, a combination using nicotinamide adenine dinucleotide as an electron acceptor is mentioned, using phosphokinase and glucose-6-phosphate dehydrogenase as an enzyme.

[0008] the biosensor possessing the electrode system by which the biosensor of this invention was formed on the insulating substrate and the insulating substrate and which contains the electrode of a pair at least, and the reaction reagent layer containing the enzyme formed in contact with the electrode system, and warming for warming a reaction reagent layer -- the section is provided. here -- warming -- the section is a heat transfer object which conducts the heat from the heat source which generates heat directly [, such as a heater,], or the heat source of the sensor exterior. especially -- warming -- the biosensor which has a heat transfer object as the section can control the temperature of a reaction reagent layer with a more sufficient precision. moreover, warming -- it is cheap and a biosensor can be manufactured rather than it arranges a heater etc. as the section. As for such a heat transfer object, it is desirable to form in the near field where the periphery of a reaction reagent layer or the reaction reagent layer of a substrate was allotted, and a reverse near field. If the heat transfer object which makes a metal a subject especially is used, high thermal conductivity is obtained and a reaction reagent layer can be warmed effectively. As a metal used for a heat transfer object, simple substances and these alloys, such as silver, aluminum, gold, nickel, and copper, are mentioned, for example.

[0009] The measuring device of this invention is the measuring device which carries out the quantum of the substrate in a specimen using the biosensor possessing an insulating substrate, the electrode system which was formed on the insulating substrate, and which contains the electrode of a pair at least, and the reaction reagent layer containing the enzyme formed in contact with the electrode system, and possesses a means impress an electrical potential difference to inter-electrode, a means detect the amount of currents which flows to an electrode, and a means warm a reaction reagent layer. in addition -- if it is the biosensor equipped with the reaction reagent layer not only the biosensor that has the above heat transfer objects but on the insulating substrate -- warming -- it is possible to warm a reaction reagent layer with a means.

[0010] desirable -- warming -- a means detects the temperature of a reaction reagent layer and has the temperature control function to hold the temperature of a reaction reagent layer to predetermined temperature. Highly precise measurement is attained by holding the temperature of a reaction reagent layer to the so-called optimum reaction temperature whenever [** / to which the specific activity of an enzyme becomes high]. Moreover, the time amount which an enzyme reaction takes can also be shortened. The PID control which used the microcomputer performs such temperature control.

[0011]

[Example] Hereafter, the desirable example of this invention is explained to a detail using a drawing. The configuration of the biosensor of this example is shown in drawing 1 and drawing 2 . As shown in (a) of drawing 1 , the electrode system of the pair which consists of a measurement pole 3 and a counter electrode 4 is formed in one field of the insulating substrate 1 which consists of polyethylene terephthalate. These are formed by the screen-stencil which used for example, conductive carbon paste. The lead section 2 was formed of the screen-stencil which used the silver paste, one lead section 2a is connected with the measurement pole 3, and lead section 2b of another side is connected with the counter electrode 4. Insulating-layer 5a is formed so that an electrode system may be surrounded, and it has covered the lead section 2 partially. Moreover, the periphery section of the measurement pole 3 is covered with insulating-layer 5b, and the area of the exposed part is prescribed by insulating-layer 5b. Although not shown in (a) of drawing 1 , as shown in drawing 2 , the reaction reagent layer 7 formed in opening of insulating-layer 5a, i.e., the front face of these electrode system, by applying a solution including an enzyme and an electron acceptor, and drying is allotted. As shown in (b) of drawing 1 , the heat transfer object 6 is formed in the field of another side of a substrate 1. The heat transfer object 6 is formed by the screen-stencil which used for example, the silver paste.

[0012] Hereafter, a glucose sensor is explained as an example of the biosensor of this invention. In the case of a glucose sensor, glucose oxidase is used as an enzyme included in the reaction reagent layer 7, and potassium ferricyanide is used as an electron acceptor, respectively. This glucose sensor is the following, and is made and used. Between the sense terminals of the pair of a measuring device, the base electrical potential difference is impressed beforehand and, as for a measuring device, wearing of a sensor is recognized by the resistance value change between terminals. Recognition of wearing of a sensor operates the heater built in equipment. The heat which the heater emitted is told to the reaction reagent layer 7 through the heat transfer object 6 of a sensor, and the temperature of the reaction reagent layer 7 rises. while a measuring device detects the temperature of a sensor -- PID control -- a sensor -- predetermined temperature, for example, 40 degrees C, -- warming -- it is set up so that it may hold. Here, a control section will notify a user of the ability to measure by a display, a beep sound, etc., if sensor temperature reaches the above-mentioned temperature. A user will supply the sample which it is going to measure to a reaction reagent layer, if this notice is received. Moreover, the sample extracted beforehand can be supplied automatically.

[0013] If a sample is supplied to a reaction reagent layer, a reaction reagent layer dissolves, and the glucose contained in the sample will oxidize by glucose oxidase, and will become a gluconic acid. At this time, the potassium ferricyanide made to live together in a reaction reagent layer is returned, and potassium ferrocyanide is generated. Equipment detects the liquid junction of the electrode system by supply of a sample, and impresses a pulse voltage to inter-electrode after predetermined time progress from sample supply by change of the electrical potential difference between terminals. Thereby,

electrolytic oxidation of the potassium ferrocyanide is carried out, and oxidation current flows in the measurement pole 3. Equipment measures the amount of currents which flows to this measurement pole 3. Since it is dependent on potassium ferrocyanide concentration, it depends for this amount of oxidation current on the glucose concentration in a sample. Equipment can judge the glucose concentration of a sample as compared with the calibration curve beforehand prepared in the acquired value.

[0014] In addition, although the heat transfer object was formed in the field opposite to the field equipped with the electrode system of an insulating substrate in the above-mentioned example, the same effectiveness is acquired, even if it forms the heat transfer object 8 in the same field as the field equipped with the electrode system of a substrate 1, or it forms the heat transfer object 9 in the side face of a substrate 1 as shown in drawing 4 as shown in drawing 3 . Moreover, although the above-mentioned example described the glucose sensor using the glucose oxidase which is a kind of an oxidoreductase as an enzyme, the same effectiveness is acquired also with the biosensor using other various enzymes.

[0015]

[Effect of the Invention] According to this invention, the assay of the substrate which can carry out the quantum of the substrate contained in a specimen with high precision, quickly, and simple can be offered, without being influenced of the temperature of the environment to measure, and the temperature of a specimen.

[Translation done.]

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TECHNICAL FIELD

[Field of the Invention] This invention relates to the assay of the substrate in a sample.

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PRIOR ART

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[0003] This biosensor is the following, and is made and used. The sample solution containing the substrate which it is going to measure is supplied to the reaction reagent layer 14. The reaction reagent layer 14 dissolves by this, and a substrate oxidizes with an oxidoreductase further. At this time, the electron acceptor included in the reaction reagent layer 14 is returned. From supply of a sample solution, an electrical potential difference is impressed between the measurement pole 12 and a counter electrode 13 after predetermined time progress, and this returned electron acceptor is oxidized electrochemically. It measures at this time, the amount of currents of oxidation current, i.e., amount, which flows to the measurement pole 12. The relational expression of substrate concentration and the amount of oxidation current is beforehand memorized as a calibration curve by the measuring device, and a measuring device computes the substrate concentration in a sample solution for the obtained amount of oxidation current to it as compared with this calibration curve. The measurement to various matter is possible for such a biosensor by choosing as arbitration the enzyme which makes the matter used as the measuring object a substrate. However, the amount of oxidation current measured as mentioned above is influenced by the degree of advance of the enzyme reaction of the sample in the time. Since advance of an enzyme reaction is greatly dependent on the temperature of a reaction place, the value of the substrate concentration obtained by the above approaches is changed with the room temperature at the time of measuring, or the temperature of a sample solution.

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EFFECT OF THE INVENTION

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] This invention aims at offering the quantum approach of the substrate which is stabilized and can carry out the quantum of the substrate concentration of a sample solution, without solving the above trouble and being influenced of environmental temperature or the temperature of a sample solution.

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MEANS

[Means for Solving the Problem] the place, i.e., the reaction reagent layer, which performs an enzyme reaction according to this invention -- fixed temperature -- warming -- the substrate and enzyme in a specimen are made to react, holding
[0006]
[Embodiment of the Invention] The electrode system by which the assay of the substrate of this invention was formed on the insulating substrate and the insulating substrate and which contains the electrode of a pair at least, the biosensor possessing the reaction reagent layer containing the enzyme formed in contact with the electrode system -- using -- a reaction reagent layer -- warming -- with the process to which supply the specimen which contains a substrate in a reaction reagent layer, and a substrate and an enzyme are made to react, holding inter-electrode -- an electrical potential difference -- impressing -- the electrode of a pair -- the process which carries out the quantum of the substrate in a specimen based on the amount of currents which flows to inner either is provided. In the assay of the substrate using an enzyme reaction, the specific activity of an enzyme is changed with the temperature of a reaction place. Therefore, the temperature of a reaction place has big effect on the precision of a quantum. If the temperature of a reaction place is low, an enzyme reaction rate will become slow, and if temperature is high, an enzyme reaction rate will tend to become quick. Then, in the case of measurement, an enzyme reaction place, i.e., a reaction reagent layer, is warmed, and specific activity of an enzyme is made high. If the specific activity of an enzyme becomes high, a reaction rate will become large and the accuracy of measurement will improve remarkably. Moreover, it also becomes possible to shorten the detection time of a sensor.
[0007] especially -- a reaction reagent layer -- specific temperature -- warming -- an enzyme reaction can always be advanced at a fixed rate, without being influenced of the environment measured by holding, or the temperature of a specimen. Thereby, the variation in the measured value resulting from a temperature gap can be controlled. Preferably, it is the temperature to which the specific activity of an enzyme becomes high about the temperature of a reaction reagent layer, for example, a glucose sensor, and when using glucose oxidase for an enzyme, it is desirable to warm at 30-50 degrees C. After the above-mentioned assay makes a substrate react with an enzyme in a reaction reagent layer, it can be used for the approach of detecting the amount of the oxidation current which impresses the suitable electrical potential difference for the matter generated by the enzyme reaction, and is then acquired, or reduction current, or its quantity of electricity. Especially, enzymes are a substrate and an oxidoreductase to which it reacts specifically, and if it uses for the assay using the biosensor of the type to which an electron acceptor is made to return while oxidizing the biosensor with which a reaction reagent layer includes an electron acceptor further, i.e., the substrate which it is going to measure by the enzyme reaction, high measurement of precision will be attained more. In addition, it is also possible to use for the approach of detecting change of the absorbance accompanying the oxidation reduction reaction of the electron acceptor which the reaction reagent layer was made containing. In this case, a combination using nicotinamide adenine dinucleotide as an electron acceptor is mentioned, using phosphokinase and glucose-6-phosphate dehydrogenase as an enzyme.

[0008] the biosensor possessing the electrode system by which the biosensor of this invention was formed on the insulating substrate and the insulating substrate and which contains the electrode of a pair at least, and the reaction reagent layer containing the enzyme formed in contact with the electrode system, and warming for warming a reaction reagent layer -- the section is provided. here -- warming -- the section is a heat transfer object which conducts the heat from the heat source which generates heat directly [, such as a heater,], or the heat source of the sensor exterior. especially -- warming -- the biosensor which has a heat transfer object as the section can control the temperature of a reaction reagent layer with a more sufficient precision. moreover, warming -- it is cheap and a biosensor can be manufactured rather than it arranges a heater etc. as the section. As for such a heat transfer object, it is desirable to form in the near field where the periphery of a reaction reagent layer or the reaction reagent layer of a substrate was allotted, and a reverse near field. If the heat transfer object which makes a metal a subject especially is used, high thermal conductivity is obtained and a reaction reagent layer can be warmed effectively. As a metal used for a heat transfer object, simple substances and these alloys, such as silver, aluminum, gold, nickel, and copper, are mentioned, for example.

[0009] The measuring device of this invention is the measuring device which carries out the quantum of the substrate in a specimen using the biosensor possessing an insulating substrate, the electrode system which was formed on the insulating substrate, and which contains the electrode of a pair at least, and the reaction reagent layer containing the enzyme formed in contact with the electrode system, and possesses a means impress an electrical potential difference to inter-electrode, a means detect the amount of currents which flows to an electrode, and a means warm a reaction reagent layer. in addition -- if it is the biosensor equipped with the reaction reagent layer not only the biosensor that has the above heat transfer objects but on the insulating substrate -- warming -- it is possible to warm a reaction reagent layer with a means.

[0010] desirable -- warming -- a means detects the temperature of a reaction reagent layer and has the temperature control function to hold the temperature of a reaction reagent layer to predetermined temperature. Highly precise measurement is attained by holding the temperature of a reaction reagent layer to the so-called optimum reaction temperature whenever [** / to which the specific activity of an enzyme becomes high]. Moreover, the time amount which an enzyme reaction takes can also be shortened. The PID control which used the microcomputer performs such temperature control.

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EXAMPLE

[Example] Hereafter, the desirable example of this invention is explained to a detail using a drawing. The configuration of the biosensor of this example is shown in drawing 1 and drawing 2 . As shown in (a) of drawing 1 , the electrode system of the pair which consists of a measurement pole 3 and a counter electrode 4 is formed in one field of the insulating substrate 1 which consists of polyethylene terephthalate. These are formed by the screen-stencil which used for example, conductive carbon paste. The lead section 2 was formed of the screen-stencil which used the silver paste, one lead section 2a is connected with the measurement pole 3, and lead section 2b of another side is connected with the counter electrode 4. Insulating-layer 5a is formed so that an electrode system may be surrounded, and it has covered the lead section 2 partially. Moreover, the periphery section of the measurement pole 3 is covered with insulating-layer 5b, and the area of the exposed part is prescribed by insulating-layer 5b. Although not shown in (a) of drawing 1 , as shown in drawing 2 , the reaction reagent layer 7 formed in opening of insulating-layer 5a, i.e., the front face of these electrode system, by applying a solution including an enzyme and an electron acceptor, and drying is allotted. As shown in (b) of drawing 1 , the heat transfer object 6 is formed in the field of another side of a substrate 1. The heat transfer object 6 is formed by the screen-stencil which used for example, the silver paste.
[0012] Hereafter, a glucose sensor is explained as an example of the biosensor of this invention. In the case of a glucose sensor, glucose oxidase is used as an enzyme included in the reaction reagent layer 7, and potassium ferricyanide is used as an electron acceptor, respectively. This glucose sensor is the following, and is made and used. Between the sense terminals of the pair of a measuring device, the base electrical potential difference is impressed beforehand and, as for a measuring device, wearing of a sensor is recognized by the resistance value change between terminals. Recognition of wearing of a sensor operates the heater built in equipment. The heat which the heater emitted is told to the reaction reagent layer 7 through the heat transfer object 6 of a sensor, and the temperature of the reaction reagent layer 7 rises. while a measuring device detects the temperature of a sensor -- PID control -- a sensor -- predetermined temperature, for example, 40 degrees C, -- warming -- it is set up so that it may hold. Here, a control section will notify a user of the ability to measure by a display, a beep sound, etc., if sensor temperature reaches the above-mentioned temperature. A user will supply the sample which it is going to measure to a reaction reagent layer, if this notice is received. Moreover, the sample extracted beforehand can be supplied automatically.
[0013] If a sample is supplied to a reaction reagent layer, a reaction reagent layer dissolves, and the glucose contained in the sample will oxidize by glucose oxidase, and will become a gluconic acid. At this time, the potassium ferricyanide made to live together in a reaction reagent layer is returned, and potassium ferrocyanide is generated. Equipment detects the liquid junction of the electrode system by supply of a sample, and impresses a pulse voltage to inter-electrode after predetermined time progress from sample supply by change of the electrical potential difference between terminals. Thereby, electrolytic oxidation of the potassium ferrocyanide is carried out, and oxidation current flows in the measurement pole 3. Equipment measures the amount of currents which flows to this measurement pole 3. Since it is dependent on potassium ferrocyanide concentration, it depends for this amount of oxidation

current on the glucose concentration in a sample. Equipment can judge the glucose concentration of a sample as compared with the calibration curve beforehand prepared in the acquired value.
[0014] In addition, although the heat transfer object was formed in the field opposite to the field equipped with the electrode system of an insulating substrate in the above-mentioned example, the same effectiveness is acquired, even if it forms the heat transfer object 8 in the same field as the field equipped with the electrode system of a substrate 1, or it forms the heat transfer object 9 in the side face of a substrate 1 as shown in drawing 4 as shown in drawing 3 . Moreover, although the above-mentioned example described the glucose sensor using the glucose oxidase which is a kind of an oxidoreductase as an enzyme, the same effectiveness is acquired also with the biosensor using other various enzymes.

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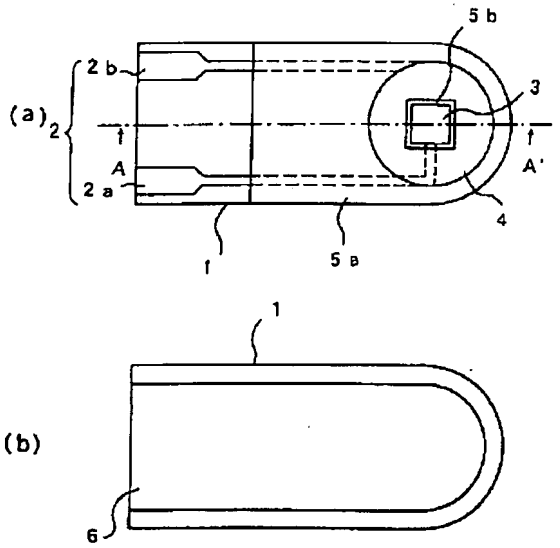
DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing the configuration of the biosensor of one example of this invention, and (a) is a top view and (b) is rear view.
[Drawing 2] It is the A-A' line sectional view of this biosensor.
[Drawing 3] It is the top view of the biosensor of other examples of this invention.
[Drawing 4] It is drawing showing the configuration of the biosensor of the example of further others of this invention, and (a) is [a top view and (c of a left side view and (b))] right side views.
[Drawing 5] It is drawing showing the configuration of the conventional biosensor, and (a) is a top view and (b) is a B-B' line sectional view.
[Description of Notations]
1 11 Substrate
2, 2a, 2b Lead section
3 12 Measurement pole
4 13 Counter electrode
5a, 5b, 15 Insulating layer
6, 8, 9 Heat transfer object
7 14 Reaction reagent layer
10 Reference Pole

[Translation done.]

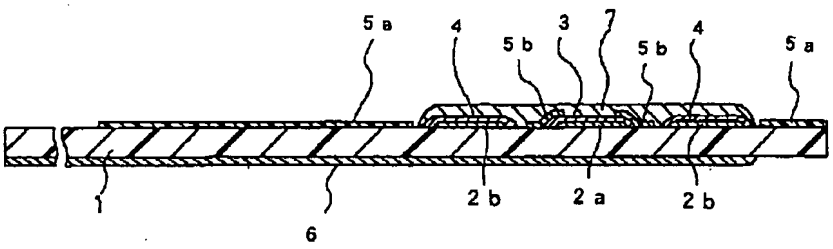
Drawing selection drawing 1



1	基板	4	対極
2	リード部	5 a、5 b、	絶縁層
3	測定極	6	伝熱体

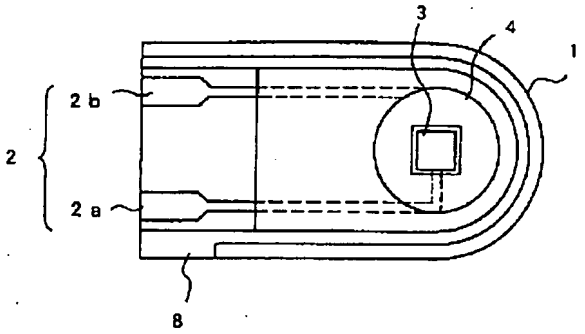
[Translation done.]

Drawing selection drawing 2



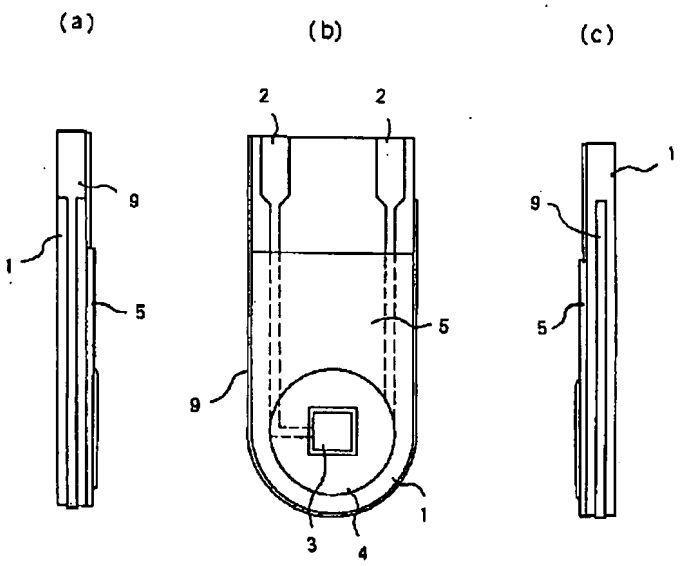
[Translation done.]

Drawing selection drawing 3



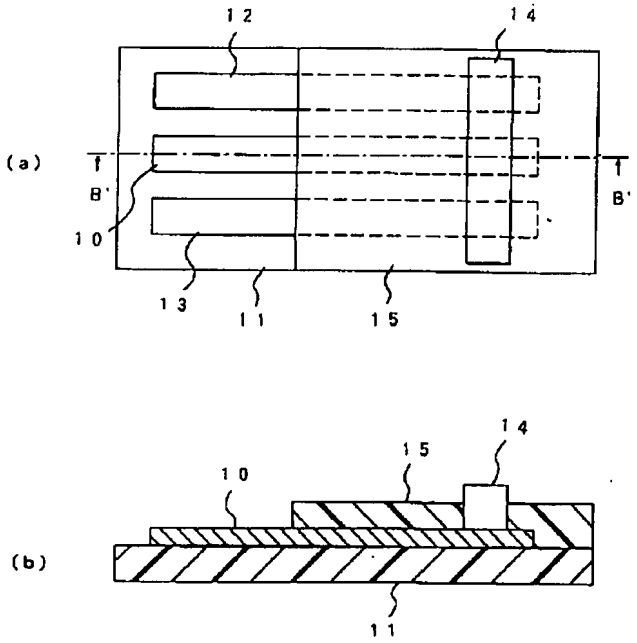
[Translation done.]

Drawing selection drawing 4



[Translation done.]

Drawing selection drawing 5



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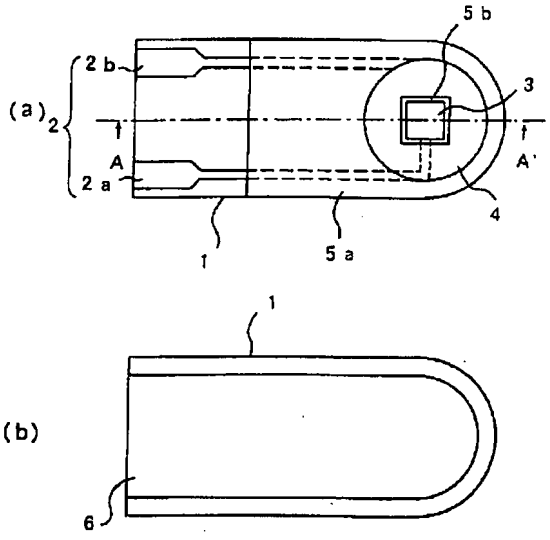
(54)【発明の名称】　基質の定量法並びにそれに用いるバイオセンサおよび測定装置

(57)【要約】
【課題】　測定する環境の温度や被検試料の温度の影響を受けずに、高精度で迅速かつ簡便に被検試料中の基質を定量することができる方法を提供する。
【解決手段】　酵素を含む反応試薬層を加温しながら、反応試薬層に基質を含む被検試料を供給して基質を反応させ、その反応量を電気化学的または光学的に検出する。



1　基板　　4　対極
2　リード部　　5 a、５ｂ、絶縁層
3　測定極　　6　伝熱体

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【特許請求の範囲】

【請求項１】　絶縁基板と、前記絶縁基板上に形成された少なくとも一対の電極を含む電極系と、前記電極系に接して形成された酵素を含む反応試薬層とを具備するバイオセンサを用い、前記反応試薬層を加温保持しながら前記反応試薬層に基質を含む被検試料を供給して前記基質と前記酵素を反応させる工程と、前記電極間に電圧を印加して前記電極のいずれか一方に流れる電流量に基づいて前記被検試料中の基質を定量する工程とを具備する基質の定量法。

【請求項２】　前記酵素が前記基質と特異的に反応する酸化還元酵素であって、前記反応試薬層がさらに電子受容体を含む請求項１記載の基質の定量法。

【請求項３】　酵素および電子受容体を含む反応試薬層を加温保持しながら、前記反応試薬層に基質を含む被検試料を供給する工程と、前記反応試薬層の吸光度の変化を検出する工程とを具備する基質の定量法。

【請求項４】　前記酵素がグルコースオキシダーゼであって、前記反応試薬層を３０～５０℃に加温保持する請求項１または３に記載の基質の定量法。

【請求項５】　絶縁基板と、前記絶縁基板上に形成された少なくとも一対の電極を含む電極系と、前記電極系に接して形成された酵素を含む反応試薬層と、前記反応試薬層を加温するための加温部とを具備するバイオセンサ。

【請求項６】　前記加温部が、外部の熱源からの熱を前記反応試薬層に伝えるための伝熱体であって、前記反応試薬層の周辺部または前記基板の反応試薬層を備えた面と反対の面に形成された請求項５記載のバイオセンサ。

【請求項７】　前記加温部が、金属を含む請求項５記載のバイオセンサ。

【請求項８】　前記酵素が前記基質と特異的に反応する酸化還元酵素であって、前記反応試薬層がさらに電子受容体を含む請求項５記載のバイオセンサ。

【請求項９】　絶縁基板と、前記絶縁基板上に形成された少なくとも一対の電極を含む電極系と、前記電極系に接して形成された酵素を含む反応試薬層とを具備するバイオセンサを用いて被検試料中の基質を定量する測定装置であって、前記電極間に電圧を印加する手段と、前記電極に流れる電流量を検出する手段と、前記反応試薬層を加温する手段とを具備する測定装置。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、試料中の基質の定量法に関するものである。

【０００２】

【従来の技術】試料中の基質を迅速かつ高精度に定量する手段としては、例えば特公平７－１１４７０５号公報に開示されたバイオセンサがある。同公報に開示されたバイオセンサを図５に示す。絶縁性の基板１１の表面に

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は測定極１２、対極１３および参照極１０からなる電極系が形成されている。これら電極系に接するように反応試薬層１４が形成されている。反応試薬層１４は、親水性高分子、酸化還元酵素および電子受容体を含む。このバイオセンサは、スクリーン印刷等の方法で基板１１上に測定極１２、対極１３および参照極１０を形成した後、所定面積の開口部を有する絶縁層１５を形成し、この開口部に反応試薬層１４を形成したものである。

【０００３】このバイオセンサは、以下のようにして用いられる。測定しようとする基質を含む試料液を反応試薬層１４に供給する。これにより反応試薬層１４が溶解し、さらに酸化還元酵素によって基質が酸化される。このとき、反応試薬層１４に含まれる電子受容体が還元される。試料液の供給より所定時間経過後、測定極１２と対極１３の間に電圧を印加して、この還元された電子受容体を電気化学的に酸化する。このとき、測定極１２に流れる電流量すなわち酸化電流量を測定する。測定装置には、あらかじめ基質濃度と酸化電流量の関係式が検量線として記憶されていて、測定装置は、得られた酸化電流量をこの検量線と比較して、試料液中の基質濃度を算出するようになっている。このようなバイオセンサは、測定対象となる物質を基質とする酵素を任意に選択することによって、様々な物質に対する測定が可能である。しかし、上記のようにして測定される酸化電流量は、その時点での試料の酵素反応の進行の度合いに影響される。酵素反応の進行は、反応場の温度に大きく依存することから、上記のような方法で得られる基質濃度の値は、測定を行う際の室温や試料液の温度によって変動する。

【０００４】

【発明が解決しようとする課題】本発明は、以上の問題点を解決するものであり、環境の温度や試料液の温度の影響を受けることなく、安定して試料液の基質濃度を定量することができる基質の定量方法を提供することを目的とする。

【０００５】

【課題を解決するための手段】本発明によると、酵素反応を行う場すなわち反応試薬層を一定の温度に加温保持しながら、被検試料中の基質と酵素を反応させる。

【０００６】

【発明の実施の形態】本発明の基質の定量法は、絶縁基板と、絶縁基板上に形成された少なくとも一対の電極を含む電極系と、電極系に接して形成された酵素を含む反応試薬層とを具備するバイオセンサを用い、反応試薬層を加温保持しながら反応試薬層に基質を含む被検試料を供給して基質と酵素を反応させる工程と、電極間に電圧を印加して一対の電極うちのいずれか一方に流れる電流量に基づいて被検試料中の基質を定量する工程とを具備する。酵素反応を利用した基質の定量法においては、酵素の比活性は、反応場の温度により変動する。したがっ

て、反応場の温度が定量の精度に大きな影響を及ぼす。反応場の温度が低いと酵素反応速度は遅くなり、温度が高いと酵素反応速度は速くなる傾向がある。そこで、測定の際に、酵素反応場すなわち反応試薬層を加温して酵素の比活性を高くする。酵素の比活性が高くなれば、反応速度が大きくなり、測定精度が著しく向上する。また、センサの検出時間を短縮することも可能になる。

【０００７】特に、反応試薬層を特定温度に加温保持することにより、測定する環境や被検試料の温度の影響を受けることなく、常に一定の速度で酵素反応を進行させることができる。これにより、温度格差に起因した測定値のバラツキを抑制することができる。好ましくは、反応試薬層の温度を、酵素の比活性が高くなる温度、例えばグルコースセンサであって酵素にグルコースオキシダーゼを用いる場合には、３０～５０℃に加温することが好ましい。上記の定量法は、反応試薬層において基質を酵素により反応させた後、酵素反応により生成された物質に適当な電圧を印加してそのときに得られる酸化電流あるいは還元電流の量、またはその電気量を検出する方法に用いることができる。特に、酵素が基質と特異的に反応する酸化還元酵素であって、反応試薬層がさらに電子受容体を含むバイオセンサ、すなわち酵素反応により測定しようとする基質を酸化させるとともに電子受容体を還元させるタイプのバイオセンサを用いた定量法に用いると、より精度の高い測定が可能になる。この他、反応試薬層に含有させた電子受容体の酸化還元反応に伴う吸光度の変化を検出する方法に用いることも可能である。この場合、例えば酵素としてホスホキナーゼおよびグルコース－６－リン酸脱水素酵素を用い、電子受容体としてニコチンアミドアデニンジヌクレオチドを用いる組み合わせが挙げられる。

【０００８】本発明のバイオセンサは、絶縁基板と、絶縁基板上に形成された少なくとも一対の電極を含む電極系と、電極系に接して形成された酵素を含む反応試薬層とを具備するバイオセンサと、反応試薬層を加温するための加温部とを具備する。ここで、加温部は、ヒータ等の直接発熱する熱源、またはセンサ外部の熱源からの熱を伝える伝熱体である。特に、加温部として伝熱体を有するバイオセンサは、反応試薬層の温度をより精度よく制御することができる。また、加温部としてヒータ等を配置するよりも安価でバイオセンサを製造することができる。このような伝熱体は、反応試薬層の周辺部または基板の反応試薬層の配された側の面と反対の側の面に形成することが好ましい。特に、金属を主体とする伝熱体を用いると、高い熱伝導率が得られ、効果的に反応試薬層を加温することができる。伝熱体に用いる金属としては、例えば、銀、アルミニウム、金、ニッケル、銅などの単体およびこれらの合金が挙げられる。

【０００９】本発明の測定装置は、絶縁基板と、絶縁基板上に形成された少なくとも一対の電極を含む電極系と、電極系に接して形成された酵素を含む反応試薬層とを具備するバイオセンサを用いて被検試料中の基質を定量する測定装置であって、電極間に電圧を印加する手段と、電極に流れる電流量を検出する手段と、反応試薬層を加温する手段とを具備する。なお、上記のような伝熱体を有するバイオセンサに限らず、絶縁基板上に反応試薬層を備えたバイオセンサであれば、加温手段によって反応試薬層を加温することが可能である。

【００１０】好ましくは、加温手段が、反応試薬層の温度を検出し、反応試薬層の温度を所定温度に保持する温度調節機能を有する。反応試薬層の温度を酵素の比活性が高くなる温度いわゆる至適反応温度に保持することで、高精度の測定が可能となる。また、酵素反応に要する時間を短縮することもできる。このような温度調節は、例えばマイクロコンピュータを用いたＰＩＤ制御により行う。

【００１１】

【実施例】以下、本発明の好ましい実施例を、図面を用いて詳細に説明する。本実施例のバイオセンサの構成を図１および図２に示す。ポリエチレンテレフタレートからなる絶縁性の基板１の一方の面には、図１の（ａ）に示すように、測定極３および対極４からなる一対の電極系が形成されている。これらは、例えば導電性カーボンペーストを用いたスクリーン印刷により形成する。リード部２は、銀ペーストを用いたスクリーン印刷により形成されたもので、一方のリード部２ａは測定極３と接続されていて、他方のリード部２ｂは対極４と接続されている。絶縁層５ａは、電極系を取り囲むように形成されていて、リード部２を部分的に覆っている。また、測定極３の周縁部は絶縁層５ｂにより覆われていて、その露出部分の面積は、絶縁層５ｂにより規定されている。図１の（ａ）には示さないが、図２に示すように、絶縁層５ａの開口部すなわちこれら電極系の表面には、酵素および電子受容体を含む溶液を塗布、乾燥することにより形成された反応試薬層７が配されている。基板１の他方の面には、図１の（ｂ）に示すように、伝熱体６が形成されている。伝熱体６は、例えば銀ペーストを用いたスクリーン印刷により形成する。

【００１２】以下、本発明のバイオセンサの一例として、グルコースセンサについて説明する。グルコースセンサの場合、反応試薬層７に含ませる酵素としてグルコースオキシダーゼを、電子受容体としてフェリシアン化カリウムをそれぞれ用いる。このグルコースセンサは、例えば以下のようにして用いられる。測定装置の一対の測定端子間には、あらかじめベース電圧が印加されていて、測定装置は、端子間の抵抗値の変化によってセンサの装着を認識するようになっている。センサの装着が認識されると、装置に内蔵されたヒータが作動する。ヒータの発した熱は、センサの伝熱体６を通じて反応試薬層７に伝えられ、反応試薬層７の温度が上昇する。測定装

置は、センサの温度を検出しながら、ＰＩＤ制御によりセンサを所定の温度例えば４０℃に加温保持するように設定されている。ここで、制御部は、センサ温度が上記温度に達すると、測定が可能であることを表示や警告音等で使用者に通知する。使用者は、この通知を受けると、測定しようとする試料を反応試薬層に供給する。また、あらかじめ採取した試料を自動的に供給するようにすることもできる。

【００１３】試料が反応試薬層に供給されると、反応試薬層が溶解し、試料に含まれていたグルコースがグルコースオキシダーゼによって酸化されてグルコン酸になる。このときに反応試薬層中に共存させておいたフェリシアン化カリウムが還元されて、フェロシアン化カリウムが生成される。装置は、端子間の電圧の変化によって試料の供給による電極系の液絡を検知し、試料供給から所定時間経過後に、電極間にパルス電圧を印加する。これにより、フェロシアン化カリウムは電解酸化され、測定極３には酸化電流が流れる。装置は、この測定極３に流れる電流量を測定する。この酸化電流量は、フェロシアン化カリウム濃度に依存することから、試料中のグルコース濃度に依存する。装置は、得られた値を、あらかじめ用意された検量線と比較して試料のグルコース濃度を判定することができる。

【００１４】なお、上記実施例では、絶縁性の基板の電極系を備えた面と反対の面に伝熱体を形成したが、図３に示すように基板１の電極系を備えた面と同一面に伝熱体８を形成したり、図４に示すように基板１の側面に伝熱体９を形成しても同様の効果が得られる。また、上記実施例では、酵素として酸化還元酵素の一種であるグルコースオキシダーゼを用いたグルコースセンサについて述べたが、その他の各種酵素を用いたバイオセンサでも同様の効果が得られる。

【００１５】

【発明の効果】本発明によると、測定する環境の温度や、被検試料の温度の影響を受けずに、高精度で迅速かつ簡便に被検試料に含まれる基質を定量することができる基質の定量法を提供することができる。

【図面の簡単な説明】

【図１】本発明の一実施例のバイオセンサの構成を示す図であり、（ａ）は平面図、（ｂ）は背面図である。

【図２】同バイオセンサのＡ－Ａ’線断面図である。

【図３】本発明の他の実施例のバイオセンサの平面図である。

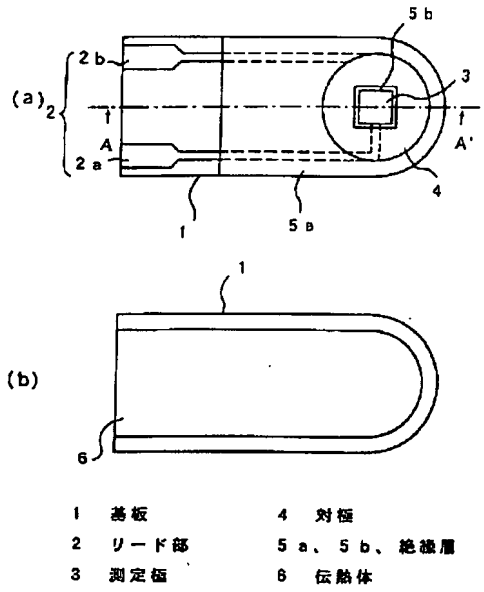
【図４】本発明のさらに他の実施例のバイオセンサの構成を示す図であり、（ａ）は左側面図、（ｂ）は平面図、（ｃ）は右側面図である。

【図５】従来のバイオセンサの構成を示す図であり、（ａ）は平面図、（ｂ）はＢ－Ｂ’線断面図である。

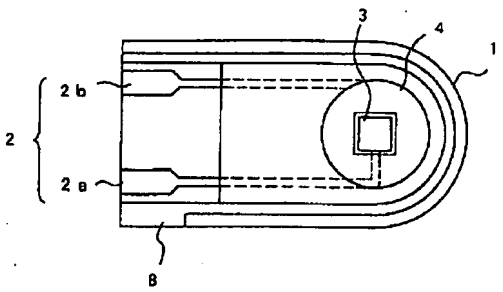
【符号の説明】

１、１１　基板
２、２ａ、２ｂ　リード部
３、１２　測定極
４、１３　対極
５ａ、５ｂ、１５　絶縁層
６、８、９　伝熱体
７、１４　反応試薬層
１０　参照極

【図１】

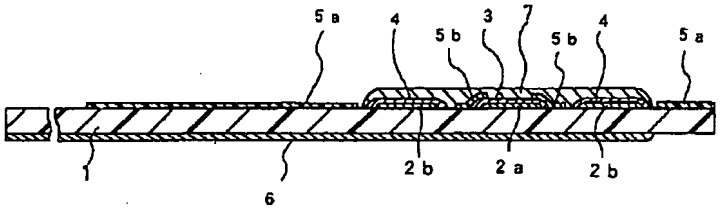


【図３】

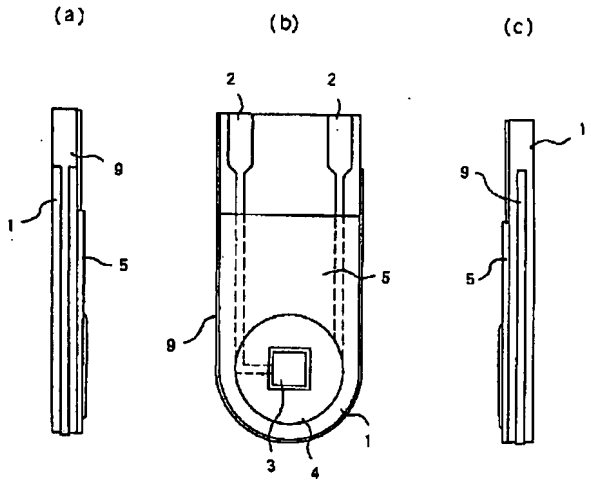


1　基板　　4　対極
2　リード部　　5a、5b、絶縁層
3　測定極　　8　伝熱体

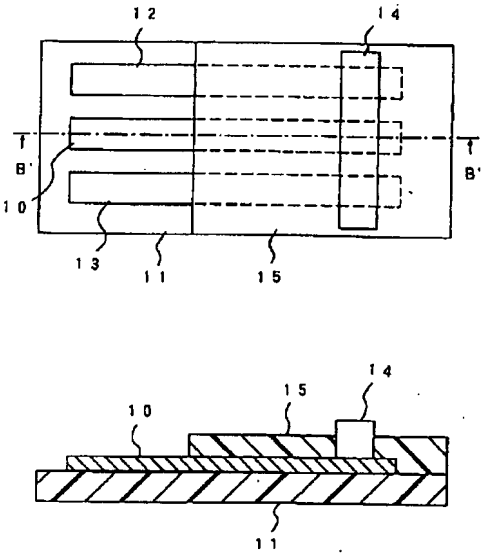
【図2】


5a
4
5b
3
7
5b
4
5a
1
6
2b
2a
2b

【図4】


(a)
(b)
(c)
9
1
5
2
2
5
9
3
4
1
1
9
5

【図5】


12
14
(a)
B
B'
B'
10
13
11
15
14
15
10
(b)
11